Effects of simultaneous polyandry on offspring fitness in an African tree frog

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In externally fertilizing animals in which females mate with multiple males at the same time (simultaneous polyandry), the possibility that females accrue genetic benefits that improve offspring viability remains largely unexamined. Here, we investigate whether simultaneous polyandry influences offspring fitness in a wild population of the Grey Foam Nest Treefrog *Chiromantis xerampelina*. Simultaneous polyandry in this frog is the most extreme reported for any vertebrate, with more than 90% of females mating with 10 or more males during the deposition of a single clutch. We compared growth (using age and size at metamorphosis as proxies) and survival of offspring produced by females that naturally mated with either 1 male (monandrous females) or 10–12 males (polyandrous females). Polyandry did not influence size or age at metamorphosis, but we found that offspring from polyandrous matings had both significantly higher mean survival and reduced variance in offspring survival. These findings implicate a genetic mechanism, but targeted cross-classified breeding experiments that control for both maternal and material effects will be required to conclusively determine whether elevated offspring viability is linked to enhanced genetic diversity, intrinsic sire effects, or genetic compatibility. Irrespective of the causation, the findings provide the first evidence that naturally formed polyandrous matings have increased offspring viability in an anuran amphibian. *Key words:* anuran amphibian, genetic benefits, offspring viability, polyandry, sexual selection. *[Behav Ecol]*

Polyandry, in which females mate with multiple males during a single reproductive cycle, is now known to be taxonomically widespread, but our understanding of why this mating system evolves is only just beginning to be understood (Birkhead 2000; Simmons 2005). In some species, polyandry appears to be driven by vigorous males forcing copulation against female interests (Thornhill 1980; Rowe 1992; Rice et al. 2006). However, in many species, polyandry may supply direct material benefits or indirect genetic benefits that increase female fitness (Zeh et al. 1997; Thom et al. 2004; Reichard et al. 2007). Early explanations for the evolution of polyandry focused on the idea that promiscuous females may directly increase their offspring production by gaining additional material benefits such as nutrient donations or paternal care (Parker 1970; Zeh and Smith 1985; Birkhead 1995). In more recent years, arguments for the evolution of polyandry have centered on the possibility that females might indirectly increase their reproductive fitness by supplying offspring with genetic benefits (Watson 1991; Jennions and Petrie 2000; Simmons 2005; Ivy 2007; McLeod and Marshall 2009).

Although a diversity of genetic benefit models for polyandry have been proposed, these generally fall into 3 broad categories: intrinsic male quality (good genes), genetic compatibility, and genetic diversity (Simmons 2005; McLeod and Marshall 2009). Under good genes models, it is argued that paternal genes influence offspring viability or attractiveness (Curtsinger 1991; Keller and Reeve 1995; Watson 1998) and that polyandrous females reduce their chance of having off-

spring sired by an inferior male (Yasui 1998; Fox and Rauter 2003; Hosken et al. 2003). This could be achieved, for example, if females preferentially use the sperm of genetically superior males (sperm selection hypothesis, Telford and Jennions 1998) or if genetically superior males produce competitively superior sperm that gain a greater share of paternity during sperm competition (good sperm hypothesis, Gage et al. 2004). In contrast, genetic compatibility models propose that offspring viability is contingent on interactions between paternal and maternal haplotypes and that polyandrous females reduce the risk of supplying offspring with incompatible genes (Zeh JA and Zeh DW 1996; Tregenza and Wedell 1997; Simmons 2005; Reichard et al. 2007). The "genetic diversity" models propose that polyandry increases female fitness either because genetically dissimilar offspring compete less intensively for limited resources (e.g., food or shelter) or because increased genetic variance cushions against fluctuating selective forces in stochastic environments (Slatkin 1974; Hosken and Blanckenhorn 1999; Forsman et al. 2007; McLeod and Marshall 2009).

Over the past 10 years, genetic benefit models have received considerable attention, and there is now convincing evidence derived from both correlational and empirical studies to suggest that polyandry might evolve as a mechanism to secure good or compatible genes (Jennions and Petrie 2000; Garcia-Gonzalez and Simmons 2005; Simmons 2005). There is also emerging evidence that benefits of polyandry can be mediated by enhanced genetic diversity (Hughes et al. 2008; McLeod and Marshall 2009). Despite this growing support for genetic benefits, our efforts to understand their influence on the evolution of polyandry have been constrained by a lack of data for animal groups that use an external mode of fertilization. Although polyandry is common in animals that use external fertilization (Brockmann et al. 1994; Halliday 1998; Taborsky 1998; Oliveira et al. 2008), evidence for genetic

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benefits has remained limited to broadcast spawning marine invertebrates (e.g., sea urchins: Metz et al. 1994; Vacquier 1998; Levitan 2002; Evans and Marshall 2005; Marshall and Evans 2005; McLeod and Marshall 2009) and fishes (Reichard et al. 2007; Kekäläinen et al. 2010). As a result, there remains a limited understanding of the indirect fitness consequences of polyandry under conditions of external fertilization.

Among anuran amphibians (frogs and toads), which rely almost exclusively on external fertilization, polyandry is relatively widespread having been reported in at least 8 species from 5 families (for references, see Byrne and Whiting 2008). In many of these species, polyandry appears to be driven by males forcing copulation while competing violently for matings, and this often leads to high costs for females (e.g., reduced fertilization success and increased risk of drowning) (D'Orgeix and Turner 1995; Halliday 1998; Byrne and Roberts 2004). However, recent quantitative genetic research using in vitro fertilization techniques in combination with crossclassified breeding designs has provided compelling evidence that embryo viability in 2 Australian frog species (Crinia georgiana and Litoria peronii) can be influenced by parental compatibility and/or intrinsic sire quality (Dziminski et al. 2008; Sherman et al. 2008a, 2008b, 2009). Taken together, the findings for C. georgiana and L. peronii suggest that genetic benefits might be playing an important role in the evolution of polyandry in frogs, but to provide added support for this inference, it needs to be examined whether fitness benefits are also evident when offspring are derived from natural matings. Such work is needed to provide insights into whether indirect benefits might be manifest under conditions where precopulatory sexual selection processes (e.g., male-male competition and mate choice) have not been experimentally excluded.

The Grey Foam Nest Treefrog Chiromantis xerampelina provides a unique opportunity to examine whether naturally formed polyandrous matings might influence offspring survival and performance in an anuran. In this frog, mating pairs spawn into foam nests constructed by females and simultaneous polyandry results from unpaired males gathering around a mating pair and releasing sperm (Kusano et al. 1991; Jennions et al. 1992). Because additional males do not directly contact a female during mating, this creates a unique situation whereby multiple males fertilize the clutch of a single female (for multiple paternity data see Jennions and Passmore 1993), without females directly engaging in multiple copulations. As such, there is no need to adopt complicated experimental designs normally required to control for female mating frequency or male mating order (Simmons 2005), and tests for the effects of polyandry on offspring viability can be made on clutches derived from naturally formed matings. Although it is important to note that correlational studies are unable to control for potentially confounding maternal effects (e.g., variance in female genetic quality), testing for an association between polyandry and offspring viability is an important first step toward elucidating whether genetic benefits have contributed to the evolution of polyandry (cf. Arnqvist and Nilsson 2000; Konior et al. 2001; Pai and Yan 2002).

In this study, we investigated whether naturally formed polyandrous matings in *C. xerampelina* have an influence on offspring growth and/or survival, and correspondingly, female reproductive success.

MATERIALS AND METHODS

Study species

Chiromantis xerampelina is a large (50–80 mm snout-vent length [SVL]) rhacophorid frog that is widely distributed in regions of mesic savanna throughout southeastern Africa (Du

Preez and Carruthers 2009). Breeding in *C. xerampelina* is prolonged, with mating activity occurring at night during the wet summer months from October to February, and is typically arboreal, with paired females constructing foam nests in tree branches overhanging water bodies (Jennions et al. 1992). In order to build their nests, females release a cloacal mucous mixed with dilute urine and whip it into a lather using the back legs (Seymour and Loveridge 1994). Females deposit their eggs into the foam nest during construction, which requires several discrete bouts of foaming to complete. After oviposition, embryos develop rapidly within the nest, and within \sim 6 days, tadpoles break free from the foam and drop into the water below, where development is then completed within \sim 6 to 8 weeks (Byrne PG, unpublished data).

Study site

The study was conducted on a natural population in Hans Merensky Nature Reserve (lat $24-25^{\circ}38'$ S, long $31-22^{\circ}40'$ E, 462 m a.s.l.) located ~70 km northeast of Tzaneen in Limpopo Province, South Africa. The study site was a large dam (Tsonga Kraal Dam) bordered by native savanna bushveld (Mucina and Rutherford 2006). The dam filled to capacity following torrential rainfall on 18 November 2006, and breeding commenced when frogs entered the site the next evening. Breeding was largely restricted to tree branches overhanging the edge of the dam but also took place on emergent vegetation within the dam. Collection of data took place between 19 November 2006 and 14 February 2007.

Behavioural observation of matings

The study site was monitored every night between 18:00 and 07:00 h, and mating frogs were located by walking the periphery of the dam and visually searching trees. When recently formed mating assemblages (i.e., prenest construction) were detected, they were video recorded under infrared light using a SONY handheld video camera (model DCR-HC42) set on night shot mode. All recordings were made at a minimum distance of ~ 4 m to reduce disturbance to mating frogs. Videos were viewed the following day, and for each mating, a male was recorded as a participant if he inserted his cloaca into a foam nest and then displayed stereotypical behaviors (e.g., muscle convulsions) associated with sperm release (cf. Byrne and Whiting 2008). After mating assemblages broke up, all the frogs involved were collected and individually contained in plastic zip lock bags. For each frog, we measured SVL to the nearest millimeter using a plastic ruler and also took a toe clip to permit individual identification. All frogs were then returned to their point of capture.

Collection of nests and offspring

We collected nests from a total of 20 matings, 10 matings that involved a single female and a single male (monandrous matings) and 10 matings that involved a single female and 10–12 males (polyandrous matings) (Figure 1). Females involved in monandrous and polyandrous matings were not significantly different in body size (mean \pm standard error SVL for monandrous females: 71.4 \pm 0.85, polyandrous females: 69.6 \pm 0.85; $t_{18} = 1.48$, P = 0.15). Nests were collected over 5 consecutive nights with exactly 4 nests (2 monandrous and 2 polyandrous) collected per night. To ensure independence, we only collected nests in which there had been no toeclipped frogs involved. In ectothermic vertebrates such as frogs, incubation conditions experienced by embryos during early development can have profound effects on phenotypic traits that impact offspring fitness (Bradford and Seymour



Figure 1

Mating in the African foam-nesting tree frog *Chiromantis xerampelina*: (a) one female and one male (monandrous mating) and (b) one female (positioned centrally) and 10 males (polyandrous mating).

1988; Shine and Harlow 1996). Therefore, to standardize developmental conditions, every nest was removed from its construction site \sim 22 to 24 h after completion and translocated to an air-conditioned room (maintained between 26-31 °C) located in the Tsonga Kraal museum, situated less than 20 m from the reservoir. To maintain a humid incubation environment, and prevent egg desiccation, each nest was completely enclosed in an aluminum wire mesh frame that was suspended within a 10-l plastic bucket, containing 5 l of fresh dam water. The bucket was then completely enclosed with a plastic bag for \sim 72 h, after which time nests were transferred to another 10-l bucket containing 5 l of fresh dam water and gently agitated in order to release tadpoles from the congealed foam. For each nest, we then used a nylon fish net to collect a random subset of exactly 200 tadpoles, which were temporarily held in 10-l plastic buckets containing 5 l of dam water.

Experimental design

Within 10 min of being counted, tadpoles from each clutch were transferred to a separate large experimental plastic pool $(110 \times 96 \times 20 \text{ cm})$, which contained ~50 l of fresh water pumped from the nearby Letaba River. The experiment involved 2 treatments (monandrous vs. polyandrous mating), and each treatment was replicated with 10 matings (experimental pools). The experimental pools (N = 20) were arranged in a straight line along the south-facing veranda of the Tsonga Kraall Museum, and to avoid spatial effects, monandrous and polyandrous pools were ordered in an alternating fashion. To protect tadpoles from predation and sun exposure, each experimental pool was covered with domestic grade shade cloth (50% UV radiation block). To prevent water fouling, every 5 days we changed approximately half of the water in each pool, and every 15 days, the pool water was completely changed.

Prior to complete water change, tadpoles from each clutch were collected and stored in separate 10-l plastic buckets so that experimental pools could be scrubbed to remove algal growth. Immediately after pools were cleaned, they were refilled with fresh water and tadpoles were returned. The entire process of cleaning and complete water change took ~ 1 h. Feeding of tadpoles coincided with water changes so that tadpoles were fed commercial aquarium grade fish flakes every 5 days, but the amount of food provided was adjusted throughout the experiment to ensure that excess food was always available. In response to tadpole growth, each tub was given 0.5 g for the first 2 cycles, 1 g for the second 2 cycles, 2 g for the third 2 cycles, 4 g for the fourth 2 cycles, and then 6 g for every remaining cycle.

Tadpole development was monitored daily. Tadpoles were removed from the experimental tubs within 24 h of them developing their front and back legs (Gosner stage 44-47) and transferred into medium sized "holding" tubs (50 \times 30×15 cm) kept in an air-conditioned room. Separate holding tubs were assigned to each experimental tub, and these tubs, which contained ~ 250 ml of water, were tilted at a 45° angle to create a small pool at one end. Tadpoles remained in these containers until they crawled out of the water (metamorphosed), which took between 5 and 7 days. The development of premetamorphic tadpoles was monitored twice a day (ca. 06:00 AM, 18:00 PM), and as soon as tadpoles metamorphosed, they were collected and weighed. The experiment ran for 90 days and was terminated only after every tub containing surviving offspring produced at least one offspring that metamorphosed. All surviving offspring were released into the dam at the sites where nests were initially collected.

Data analysis

To test whether polyandry influenced offspring viability, we measured 1) mean offspring performance; 2) best offspring performance; and 3) variation in offspring performance. We quantified offspring performance using the following variables: 1) percent offspring survival; 2) percent offspring that metamorphosed; 3) time to metamorphosis; and 4) body size at metamorphosis. Mean offspring performance was used to detect any viability benefits that arise because a clutch gains increased average performance due to fertilization by one or more outstanding sires. Best offspring performance was used to detect if polyandrous females occasionally have offspring sired by males carrying extremely high-quality genes, which can be manifested by females producing a small subset of superior offspring. Intraclutch variance was measured to test if polyandry reduces the variance in offspring performance, which is to be expected if polyandry ameliorates fitness effects resulting from fertilization by males of both extremely high and extremely low genetic quality. These 3 fitness measures have been used previously to test for genetic benefits of polyandry in frogs (Byrne and Roberts 2000).

For analysis of mean offspring performance, we compared monandrous versus polyandrous clutches for 1) the percentage of offspring that survived the duration of the experiment (90 days); 2) the percentage of offspring that successfully metamorphosed; 3) time taken to metamorphose; and 4) body size at metamorphosis. Differences in mean performance were compared using one-way analyses of variance (ANOVAs), unless standard deviations between treatments were unequal, in which case a Welch ANOVA was used. In each model, factors (fixed) were mating type (monogamous vs. polyandrous) and dependent variables were the viability measures (% survival, % clutch metamorphosed, time to metamorphosis, body size at metamorphosis).



Figure 2



For analysis of best offspring performance, we scored each clutch for the shortest time taken by an offspring to metamorphose and the largest body size (weight) at which an offspring metamorphosed. Time to metamorphosis and body size at metamorphosis are commonly used measures of fitness for frogs that breed in ephemeral pools, with the expectation being that fitter offspring are those that metamorphose more rapidly or at a larger body size (Byrne and Roberts 2000). Comparisons between monandrous and polyandrous clutches were made using one-way ANOVAs.

To test for differences between monandrous and polyandrous clutches for intraclutch variance in 1) percent offspring survival; 2) percent offspring metamorphosed; 3) fastest offspring to metamorphose; and 4) largest offspring to metamorphose, we used Levene's tests. We also calculated coefficients of variation (McVey and Smittle 1984) for 1) the average time taken to metamorphose and 2) the average body size at metamorphosis. Differences in coefficients of variation between monandrous and polyandrous clutches were analyzed using one-way ANOVAs. All statistical analyses were performed using JMP statistical Software.

RESULTS

Mean offspring performance

Mean offspring survival was over 20% higher in clutches derived from polyandrous matings compared with clutches de-

Table 1

Compariso	n between	the viability o	f Chiroman	tis xerampe	elina
offspring d	erived from	n monandrou	s versus po	lyandrous	clutches

	Treatment		
Fitness measure	Monandry	Polyandry	
Percent metamorphosed Time to metamorphosis	$\begin{array}{l} 28.95 \pm 6.15 \ (10) \\ 73.14 \pm 3.25 \ (8) \end{array}$	$\begin{array}{c} 40.9 \pm 6.15 \ (10) \\ 81.51 \pm 2.90 \ (10) \end{array}$	
Size at metamorphosis (g)	0.70 ± 0.02 (8)	$0.72 \pm 0.02 (10)$	

Values represent mean \pm standard error; sample sizes (number of clutches) are in parentheses. Sample sizes are lower for monandrous clutches because 2 clutches completely failed.

rived from monandrous mating (Welch ANOVA: $F_{1,19} = 6.36$, P = 0.02; Figure 2). The mean percentage of offspring that successfully metamorphosed was not significantly different between polyandrous and monandrous matings (ANOVA: $F_{1,19} = 1.88$, P = 0.18; Table 1). Neither average time taken to metamorphose (ANOVA: $F_{1,17} = 3.68$, P = 0.07; Table 1) nor the average body weight at metamorphosis (ANOVA: $F_{1,17} = 0.40$, P = 0.53, body weight; Table 1) was significantly different for polyandrous and monandrous clutches.

Best offspring performance

Neither the shortest time to metamorphosis (ANOVA: $F_{1,17} = 1.26$, P = 0.27) nor the largest offspring to metamorphose (ANOVA: $F_{1,17} = 1.39$, P = 0.25) were significantly different between monandrous and polyandrous clutches (Table 2).

Variance in offspring performance

Variance in the percentage of offspring that survived was significantly reduced in polyandrous clutches (Levene's test, F = 4.71, P < 0.05). Although percent survival in polyandrous clutches ranged between 42% and 68%, monandrous clutches displayed both the lowest (0%) and the highest (77%) survival. For the monandrous clutches (n = 2) that had no offspring survival (0%), mortality occurred steadily over the experimental period, and clutch failure was not linked to an unexpected event. Variance in the percentage of offspring that metamorphosed was not significantly different between polyandrous and monandrous matings (Levene's test: F =1.93, P = 0.1). For best performers, the variance between mating types for the fastest offspring to metamorphose (Levene's test: F = 0.77, P = 0.39) and the largest offspring to metamorphose (Levene's test: F = 0.001, P = 0.97) were not significantly different. Analysis of coefficients of variation showed no significant difference between polyandrous and monandrous clutches for mean time to reach metamorphosis (ANOVA, $F_{1,17} = 0.93$, P = 0.34) or mean weight at metamorphosis (ANOVA, $F_{1,17} = 1.8$, P = 0.19; Table 3).

DISCUSSION

Our results showed that offspring from polyandrous matings did not reach metamorphosis earlier, were no more likely to reach metamorphosis, and were no heavier than offspring from monandrous couplings. These results therefore provide no evidence that polyandry in *C. xerampelina* supplies offspring with growth or developmental advantages. Our results do show, however, that polyandrous clutches experienced a significant, and substantial (>20%), increase in offspring survival.

Table 2

Comparison between 2 performance measures for *Chiromantis xerampelina* offspring derived from monandrous versus polyandrous clutches

	Treatment			
Fitness measure	Monandry	Polyandry		
Minimum time (days) to metamorphosis	56.25 ± 2.61 (8)	$60.2 \pm 2.34 (10)$		
Maximum size (g) at metamorphosis	0.93 ± 0.03 (8)	$1.00 \pm 0.03 (10)$		

Values represent mean \pm standard error; sample sizes (number of clutches) are in parentheses. Sample sizes are lower for monandrous clutches because 2 clutches completely failed.

Table 3

Comparison between the coefficients of variation for 2 performance measures for *Chiromantis xerampelina* offspring from monandrous versus polyandrous clutches

	Treatment			
Fitness measure	Monandry	Polyandry		
Time to metamorphosis (days)	13.31 ± 0.84 (8)	$12.22 \pm 0.75 (10)$		
Size at metamorphosis (g)	20.24 ± 1.23 (8)	$18.01 \pm 1.10 \ (10)$		

Values represent mean \pm standard error; sample sizes (number of clutches) are in parentheses. Sample sizes are lower for monandrous clutches because 2 clutches completely failed.

As with other correlational studies designed to investigate the genetic benefits of polyandry (cf. Arnqvist and Nilsson 2000), it is possible that the survival benefit we have reported might be explained by maternal effects (see Simmons 2005). For example, females that mated monandrously may have differed in quality or habitat choice and males of certain quality may have avoided or failed to detect these females. As a result, differences in offspring quality may have reflected differences in dam quality rather than the number of males involved in a mating. We did not see any obvious differences in nest site selection between monandrous and polyandrous females, and we also found no evidence for a difference in body size between monandrous and polyandrous females. Even so, we cannot dismiss the possibility of maternal effects until additional quantitative genetic experiments have been conducted (Sheldon 2000; Simmons 2005).

It is also possible that the survival benefit we have reported might be explained by material benefits. Specifically, if polyandrous matings receive larger total ejaculate volumes, eggs may have received added nutrients (transmitted via the seminal fluid) that increased the availability of yolk for posthatching development. In turn, this could have elevated larval survival (Kekäläinen et al. 2010). Furthermore, if sperm competition arising from polyandrous matings leads to eggs being exposed to increased concentrations of seminal fluid steroids (cf. McCormick 1999), this could have accelerated rates of yolk metabolism, with subsequent effects on posthatching larval growth rates, and ultimately, offspring fitness. The potential for such postcopualtory effects has recently been considered in a study of genetic benefits of polyandry in an externally fertilizing fish (Arctic charr).

An alternative explanation for our findings is that polyandrous matings resulted in offspring receiving combinations of genes that increased their viability. Several genetic mechanisms might have underpinned the survival benefit we have reported. One possibility is that mean offspring survival was elevated due to an increase in the genetic diversity of a clutch (genetic-diversity hypothesis). Theoretically, the production of more genetically diverse offspring may either reduce the intensity of sibling competition for resources (McLeod and Marshall 2009) or cushion against unstable developmental environments (Fox and Rauter 2003). At present, nothing is known about kin recognition and larval competition in C. xerampelina, but if monandrous clutches experienced more intensive competition (e.g., Rana arvalia, Shvarts and Pyastolova 1970), they would be expected to have consistently incurred lower survival. Instead, monandrous clutches displayed both the lowest and the highest survival. This finding provides no evidence for reduced competition among halfsiblings but coupled with the result that polyandrous clutches experienced a significant reduction in variance in survival, this is the outcome expected if polyandry operates as a genetic bet-hedge against unstable developmental conditions (Fox and Rauter 2003).

In C. xerampelina, breeding typically takes place in highly stochastic environments because tadpoles develop in ephemeral summer pools that are prone to extreme and unpredictable fluctuations in biotic and abiotic factors (e.g., predation, temperature, food availability, and water levels) known to influence anuran larval survival (Jennions and Passmore 1993; Alford 1999). In other frogs species that breed under similarly heterogeneous conditions, bet-hedging mechanisms have been shown to underlie the evolution of various life-history strategies (e.g., staggering of eggs, variance in body size, and asynchronous hatching) that reduce the risk of reproductive failure (e.g., (Lips 2001; Thumm and Mahony 2002; Byrne and Keogh 2009). It has also now been established that larval life-history traits in frogs can be paternally inherited (Laurila et al. 2002). Therefore, it seems plausible that polyandrous matings in C. xerampelina may result in the production of offspring with a range of behavioral, morphological, and physiological life-history attributes that ameliorate the negative effects of environmental stochasticity. However, whether or not the viability effects, we have reported are actually linked to genetic diversity mechanisms will remain uncertain until 2 further lines of evidence have been attained. First, it will need to be established that polyandry leads to a significant increase in genetic variability (Hosken and Blanckenhorn 1999) and second, it needs to be empirically demonstrated that fitness effects of male genotypes vary under different environmental conditions (i.e., genotype \times environment interactions) (Fox and Rauter 2003).

Theoretically, polyandry could also have resulted in reduced variance in offspring survival if multiple paternity functioned as a genetic bet-hedge against faulty sire choice. Specifically, polyandry may have led to an increase in mean offspring survival by ameliorating fitness costs associated with high variance in male genetic quality (additive genetic effects) or compatibility (nonadditive genetic effects) (Zeh JA and Zeh DW 1997; Yasui 1998; Hosken and Blanckenhorn 1999; Tregenza and Wedell 2002; Fox and Rauter 2003; Hosken et al. 2003). Such genetic safeguards might be important in C. xerampelina because female mate choice has the potential to be compromised due to forced copulation by indiscriminate males. Because breeding is arboreal and females can only reach suitable nesting sites by climbing the trunks of certain trees, their movements are very predictable, which makes them susceptible to interception by unpaired males that wait at the base of trees (Byrne PG, personal communication). Consequently, female C. xerampelina may often risk mating with genetically undesirable males. We have no data on the heritability of fitness related traits in C. xerampelina, but given the findings of our past work that males in this species vary greatly in phenotypic quality (see Byrne and Whiting 2008), males should also be expected to display significant variation in genotypic quality. To disentangle the relative importance of additive and nonadditive effects on offspring viability in C. xerampelina, while controlling for potential maternal and material confounds (see above), it will be necessary to run quantitative cross-classified breeding experiments (e.g., North Carolina type II designs) (Ivy 2007).

Regardless of the causation of elevated offspring survival in polyandrous matings, our study provides some valuable insights into the evolution of polyandry in anuran amphibians. Prior to our study, examination of indirect benefits of polyandry in anurans had only been attempted using artificial reproductive procedures that involved controlled, but random allocation of mating partners (Dziminski et al. 2008; Sherman et al. 2008a, 2008b, 2009). However, random assignment of

mating partners eliminates any effects of precopulatory sexual selection on offspring fitness. By conducting experiments using naturally formed matings, our study provides some evidence that polyandry in anurans has the potential to generate indirect genetic benefits in nature. Moreover, because genetic benefits in frogs have only been previously reported in one other species (C. georgiana) in which polyandry is the confirmed mating system (Dziminski et al. 2008), our results also draw attention to the possibility that genetic benefits might be widespread in anurans. Quacking frogs (C. georgiana) and foam-nesting tree frogs (C. xerampelina) differ markedly in breeding ecology (e.g., aquatic vs. arboreal breeding) and represent separate anuran clades (the Hyloidea and the Ranoidea). Therefore, genetic benefits might be a pervasive benefit underlying the evolution of polyandry in anuran amphibians. If future work confirms this inference, it would challenge a long-standing belief that animals with external reproduction are much less likely to gain genetic benefits from polyandry due to their lack of sperm storage and limited mechanisms for postcopulatory paternity manipulation (Sivinski 1984; Simmons 2005; Zeh JA and Zeh DW 2008; Simmons et al. 2009).

In conclusion, our results indicate that polyandry in the foam-nesting tree frog *C. xerampelina* results in an increase in mean offspring survival as well as reduced variance in off-spring survival. These findings implicate a genetic mechanism, but targeted quantitative experiments that control for maternal effects and material effects (arising from variable sperm volumes) will be required to elucidate whether elevated offspring viability is linked to enhanced genetic diversity, intrinsic sire effects, or genetic compatibility. Our study is the first evidence that naturally formed polyandrous matings elevate offspring viability in an anuran amphibian and contributes to a growing literature indicating that nonmaterial benefits might explain the evolution of polyandry in animals that use either internal or external modes of reproduction.

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